Claim rejections under 35 U.S.C. § 112, first paragraph

Claims 1, 3-6, 10-11, 19, 21-23, 30-33, 37, 38, and 46-50 are rejected under 35 U.S.C. 112, first paragraph. Applicants submit that the present claim amendments and cancellations render this ground of rejection moot. However, in as much as this ground of rejection can be applied to the amended claims, applicants submit the forgoing comments.

The Examiner sated that the specification "does not reasonably provide enablement for methods of modulating apoptosis in a cell in a mammal comprising administering the mammal the agent putrescine." The Examiner further notes that role of polyamines (including putrescine) in modulating apoptosis in cells is highly complex and ambiguous, and goes on to cite various references where polyamines are shown to delay the onset of apoptosis as well as references where polyamines facilitate the induction of apoptosis. Although the Examiner is correct in that polyamines appear to be "ambiguous" at first glance, upon a closer study they are not.

First, as a review, the reaction that DHS catalyzes involves transferring a butylamine residue from spermidine to a conserved lysine on inactive eIF-5A, converting the lysine to deoxyhypusine. *See* page 4 of the present specification. A second enzyme, deoxyhypusine hydroxylase, converts deoxyhypusine to hypusine. *See id.* The hypusinated eIF-5A is the activated form of eIF-5A, which is believed to shuttle mRNAs from the nucleus to the cytoplasm. *See* page 5. Thus, effecting the ability of DHS to convert lysine to deoxyhypusine on the inactive eIF-5A reduces or inhibits the amount of eIF-5A capable of activation by deoxyhypusine hydroxlylase (thus ultimately reducing the amount eliminating active Factor 5A). *See* page 25.

Studies indicate that apoptosis can be controlled by some degree by targeting the core components of the cell death machinery. *See* page 10. The present inventors have discovered a

key protein in cell death machinery and have identified a novel isoform of eIF-5a, which they have named apoptosis-induced eIF-5A. Apoptosis-induced eIF-5A is up-regulated immediately before the induction of apoptosis, and thus is a suitable target for regulation of apoptosis, since apoptosis-induced eIF-5A acts in the post transcriptional regulation of downstream effectors and transcription factors involved in the apoptotic pathway. *See* page 11, page 39, figures 14 and 17 showing that the apoptosis-induced eIF-5A and DHS genes of the invention are up-regulated during apoptosis. Thus, by reducing the level or amount of apoptosis-induced DHS or by reducing its effectivity, the amount of acitve apoptosis-induced eIF-5A is reduced in a cell. One may then modulate the apoptotic pathways triggered by active apoptosis-induced eIF-5A. *See* page 25.

The activation of apoptosis-induced eIF5A by apoptosis-induced DHS can be reduced or blocked by administering chemical inhibitors of the DHS reaction. *See* page 42. Figures 1 and 2 show that the onset of DNA laddering reflecting apoptosis is delayed in rat corpus luteum when the animals are treated with spermidine, an inhibitor of the DHS reaction after induction of apoptosis by injection of prostaglandin $F_{2\alpha}$. *See* page 27 and 42.

Polyamines and their analogues inhibit the DHS reaction by acting as competitive inhibitors for the active site on DHS. Spermidine analogs have been successfully used to inhibit deoxyhypusine synthase *in vitro*, as well as to inhibit the formation of hypusine *in vivo*, which is accompanied by an inhibition of protein synthesis and cell growth. *See* page 6. Further, polyamines inhibit senescence, a form of programmed cell death, of plant tissues. Spermidine and putrescine have been shown to delay post-harvest senescence of carnation flowers

anddetached radish leaves. *See* page 7. Exogenous spermidine also inhibits the DHS reaction through substrate inhibition.

The finding that exogenous polyamines both inhibit and promote apoptosis reflects the fact that, depending on the level applied, they can either inhibit the DHS reaction leading to the activation of Factor 5A and hence impede apoptosis, or induce apoptosis by reason of being toxic. *See* Jakus *et al.* (1993), *J. Biol. Chem.*, 268, 13153-13159. All polyamines and their analogues are toxic at high concentration and are unable to induce apoptosis. *See* Tome, *Biochem J.*, (1997), 328, 847-854). This occurs despite their ability to inhibit activation of the apoptosis specific isoform of factor 5A for two reasons. First, activated factor 5A has a long half life and depletion of activated apoptosis-induced Factor 5A arising from inhibition of DHS activity may not occur in time to block apoptosis caused by the toxic affects of spermidine. *See* Torrelio *et al.* (1987) *Biochem. Biophys. Res. Commun.*, 145, 1335-1341; Dou & Chen, *Biochim. Biophys. Acta*, (1990) 1036, 128-137. Second, polyamines are competitive inhibitors of the DHS reaction and hence not likely to completely block the reaction even at high concentrations that are toxic.

The Examples of the present invention teach the administration of spermidine (a polyamine) to reduce apoptosis in cells. In example 5, apoptosis is inhibited in corpus luteal cells be treatment of such cells with spermidine. The degree of apoptosis was measured by DNA laddering experiments. Further in example 6, apoptosis of corpus luteum was inhibited *in vivo* in a mammal. Thus, applicants submit that there is indeed guidance in the specification as to administering a polyamine to a mammal to inhibit apoptosis. In addition to the Examples, the specification provides further guidance pertaining to the administration of polyamines to inhibit

apoptosis. On page 43, modes of administration are discussed. Further on page 44, Table 1 shows toxic levels of various compounds contemplated in the present invention.

Thus, applicants respectfully submit that given the knowledge of one skilled in the art, in combination with the teaching in the present application, the presently pending claims are enabled. Accordingly, applicants respectfully request withdrawal of this ground rejection

Claim rejections under 35 U.S.C. § 112, first paragraph

Claims 4, 10, 11, 22, 23, and 31 are rejected under 35 U.S.C. 112, first paragraph. Applicants submit that the present claim amendments and cancellations render this ground of rejection moot. Accordingly, applicants request that this ground of rejection be removed.

Claim rejections under 35 U.S.C. § 102(b)

Claims 1, 2, 3, 6, 19, 20 and 21 are rejected under 35 U.S.C. § 102(b) as being anticipated by Tome et al., *Biochem. J.*, 1997, 328, 847-854. Applicants respectfully submit that the Tome paper refers to cell division factor 5A and not the apoptosis-induced factor 5A, which is the subject of the present invention. In Tome, they are observing, upon addition of putrescine, a decrease in hypusination of an isoform of eIF-5A that controls cell division. See page 853, column 2, 3rd paragraph and column 3, last paragraph. In column 1, page 853, Tome makes the point that the excess putrescine triggers apoptosis by reason of being toxic. The Tome paper discusses inhibition of cell proliferation by the addition of putrescine because the cell division eIF-5A is being inhibited. Apoptosis is due to the toxicity of high putrescine levels. This paper does not teach nor suggest the presence of a second isoform of eIF-5A, the apoptosis-induced

eIF-5A (the subject matter of the present invention). Applicants thus, respectfully submit that the Tome reference does not anticipated the claims and accordingly ask that this ground of rejection be withdrawn.

Claim rejections under 35 U.S.C. § 102(b)

Claims 1, 2, 5, 6, 19, and 20 are rejected under 35 U.S.C. § 102(b) as being anticipated by Tome et al., *Biol. Signals*, 1997, 6, 150-156. Similar to the arguments made above, this Tome reference does not teach nor suggest the isoform of eIF-5A (apoptosis-induced eIF-5A). Tome discusses another isoform of factor 5A involved in cell division. Applicants thus respectfully submit that the Tome reference does not anticipate the claims and accordingly request withdrawal of this ground of rejection.

CONCLUSION

Applicants submit that the claims are in condition for allowance. Applicants hereby petition for a three month extension of time and authorize the Commissioner to charge the requisite fee for such extension as well as any other fee due or credit any overpayment arising

from this communication to Deposit Account No. 11-0600.

Respectfully submitted,

Date: 4/30/03

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MARKED UP VERSION TO SHOW CHANGES MADE

1. (Amended) A method for inhibiting or delaying [modulating] apoptosis in a cell, comprising [the step of] administering to said cell an agent that [inhibits apoptosis-induced eIF-5A function in said cell] is capable of inhibiting an apoptosis-induced DHS catalyzed chemical reaction, wherein said inhibiting apoptosis-induced DHS catalyzed chemical reaction reduces levels of activated apoptosis-induced eIF-5A or inhibits activation of apoptosis-induced eIF-5A; and wherein said reduction of apoptosis-induced eIF-5A or inhibition of activation of apoptosis-induced eIF-5A inhibits or delays apoptosis.

2. The method of claim 1, wherein said administering is performed *in vitro*.

3. The method of claim 1, wherein said administering is performed *in vivo*.

Cancel 4. The method of claim 1, wherein said agent inhibits transcription of an apoptosis-induced eIF-5A gene.

Cancel 5. The method of claim 1, wherein said agent inhibits translation of an apoptosis-induced eIF-5A gene transcript.

Cancel 6. The method of claim 1, wherein said agent inhibits activation of an apoptosis-induced eIF-5A protein.

Cancel 10. The method of claim 4, wherein said agent comprises a chemical or drug capable of inhibiting activation of an apoptosis-induced eIF-5A protein by apoptosis-induced DHS.

11. (Amended) The method of claim [10] 1, wherein said chemical or drug comprises spermidine, 1,3-Diamino-propane, 1,4-Diamino-butane (putrescine), 1,7-Diamino-heptane, or 1,8-Diamino-octane.

Cancel 19. A method for modulating apoptosis in a cell comprising the step of administering to said cell an agent that inhibits apoptosis-induced DHS function in said cell.

Cancel 20. The method of claim 19, wherein said administering is performed *in vitro*.

Cancel 21. The method of claim 19, wherein said administering is performed *in vivo*.

Cancel 22. The method of claim 19, wherein said agent inhibits transcription of an apoptosis-induced DHS gene.

Cancel 23. The method of claim 19, wherein said agent inhibits translation of an apoptosis-induced DHS gene transcript.

Cancel 31. The method of claim 30, wherein said agent inhibits transcription of an apoptosis-induced eIF-5A gene in said target cells of said mammal.

Cancel 32. The method of claim 30, wherein said agent inhibits translation of an apoptosis-induced eIF-5A gene transcript in said target cells of said mammal.

Cancel 33. The method of claim 30, wherein said agent inhibits activation of an apoptosis-induced eIF-5A protein in said target cells of said mammal.

Cancel 37. The method of claim 33, wherein said agent comprises a chemical or drug capable of inhibiting activation of an apoptosis-induced eIF-5A protein by an apoptosis-induced DHS protein in said target cells of said mammal.

Cancel 38. The method of claim 37, wherein said chemical or drug comprises spermidine, 1,3-Diamino-propane, 1,4-Diamino-butane (putrescine), 1,7-Diamino-heptane, or 1,8-Diamino-octane.

46. (Amended) The method of claim [30] 1, wherein said mammal is a human.

47. (Amended) The method of claim [30] 1, wherein said administration is by intraperitoneal injection.

Cancel 48. A method for modulating apoptosis in a mammal comprising the step of administering to said mammal an agent that inhibits apoptosis-induced DHS function in target cells of said mammal.

Cancel 49. The method of claim 48, wherein said agent inhibits transcription of an apoptosis-induced DHS gene.

Cancel 50. The method of claim 48, wherein said agent inhibits translation of an apoptosis-induced DHS gene transcript.

51. (New) A method for inhibiting or suppressing activation of apoptosis-induced eIF-5A comprising administering an agent that is capable of inhibiting DHS catalyzed chemical reactions, wherein the inhibiting apoptosis-induced DHS catalyzed chemical reactions inhibits or reduces an apoptosis cascade, said cascade comprising transferring a 4-aminobutyl residue from a spermidine to a ε-amino group of a conserved lysine on an inactive Factor 5A, said transferring converting the lysine to a deoxyhypusine, and wherein a deoxyhypusine hydroxylase converts the deoxyhypusine to hypusine, and wherein inhibition or reduction of said apoptosis cascade reduces an amount of activated apoptosis-induced eIF-5A or inhibits activation of apoptosis-induced eIF-5A.